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TITLE: Gene expression library produced from DNA from uncultivated microorganisms and methods for making the same

DATE-ISSUED: August 28, 2001

## INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/4; 435/183, 435/6

## CLAIMS:

What is claimed is:

1. A method for identifying an enzymatic activity of interest comprising:

culturing a gene expression library comprising a pool of expression constructs, each expression construct comprising a vector containing one or more cDNA or genomic DNA fragments, wherein the cDNA or genomic DNA fragments in the pool of expression constructs are derived from a plurality of species of donor eucaryotic organisms, and wherein the cDNA or genomic DNA fragments are each operably-associated with one or more regulatory regions that drives expression of genes encoded by the cDNA or genomic DNA fragments in an appropriate host cell; and

detecting the enzymatic activity encoded by this cDNA or genomic DNA fragments.

2. The method of claim 1, wherein the enzytatic activity is selected from the group consisting of oxidoreductase, transferase, hydrolase, lyase, isomerase, and ligase activity.

3. The method of claim 1, wherein the donor eukaryotic organisms are microorganisms.

4. The method of claim 3, wherein the microcirlganisms are derived from an environmental sample.

5. The method of claim 3, wherein the microorganisms are a mixed population of uncultured organisms.

6. The method of claim 1, wherein the organisms are fungi.

7. The method of claim 1, wherein the organisms are algae.

8. The method of claim 1, wherein the organisms are protozoan.

9. The method of claim 4, wherein the organisms are extremophiles.

10. The method of claim 9, wherein the organisms ate therimophiles, hyperthermophiles, psychrophiles, or psychrotrophs.

11. The method of claim 1, wherein the host cell is a bacterial cell.

12. The method of claim 11, wherein the bacterial cell is an E. coli, Bacillus, Streptomyces, or Salmonella typhimurium cell.

13. The method of claim 1, wherein the host cell is a fungal cell.

14. The method of claim 13, wherein the fungal cell is a yeast cell.

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15. The method of claim 1, wherein the host cell is a *Drosophila S2* or a *Spodoptera S9* cell.
16. The method of claim 1, wherein the host cell is an animal cell.
17. The method of claim 16, wherein the animal cell is a *CHO*, *COS* or *Bowes melanoma* cell.
18. The method of claim 1, wherein the host organism is a plant cell.
19. A method for identifying an enzymatic activity of interest comprising:

culturing a gene expression library comprising a pool of expression constructs, each expression construct comprising a vector containing one or more cDNA or genomic DNA fragments, wherein the cDNA or genomic DNA fragments in the pool of expression constructs are derived from a plurality of species of donor organisms, and wherein the cDNA or genomic DNA fragments are each operably-associated with one or more regulatory regions that drives expression of genes encoded by the cDNA or genomic DNA fragments in an appropriate host organism, wherein the host cell is a bacterial cell; and

detecting the enzymatic activity encoded by the cDNA or genomic DNA fragments.

20. A method for identifying an enzymatic activity of interest comprising:

culturing a gene expression library comprising a pool of expression constructs, each expression construct comprising a vector containing one or more cDNA or genomic DNA fragments, wherein the cDNA or genomic DNA fragments in the pool of expression constructs are derived from a plurality of species of donor organisms, and wherein the cDNA or genomic DNA fragments are each operably-associated with one or more regulatory regions that drives expression of genes encoded by the cDNA or genomic DNA fragments in an appropriate host organism, wherein the host cell is a fungal cell; and

detecting the enzymatic activity encoded by the cDNA or genomic DNA fragments.

21. A method for identifying an enzymatic activity of interest comprising:

culturing a gene expression library comprising a pool of expression constructs, each expression construct comprising a vector containing one or more cDNA or genomic DNA fragments, wherein the cDNA or genomic DNA fragments in the pool of expression constructs are derived from a plurality of species of donor organisms, and wherein the cDNA or genomic DNA fragments are each operably-associated with one or more regulatory regions that drives expression of genes encoded by the cDNA or genomic DNA fragments in an appropriate host organism, wherein the host cell is a plant cell; and

detecting the enzymatic activity encoded by the cDNA or genomic DNA fragments.

22. A method for identifying an enzymatic activity of interest comprising:

culturing a gene expression library comprising a pool of expression constructs, each expression construct comprising a vector containing one or more cDNA or genomic DNA fragments, wherein the cDNA or genomic DNA fragments in the pool of expression constructs are derived from a plurality of species of donor organisms, and wherein the cDNA or genomic DNA fragments are each operably-associated with one or more regulatory regions that drives expression of genes encoded by the cDNA or genomic DNA fragments in an appropriate host organism, wherein the host cell is an animal cell; and detecting the enzymatic activity encoded by the cDNA or genomic DNA fragments.